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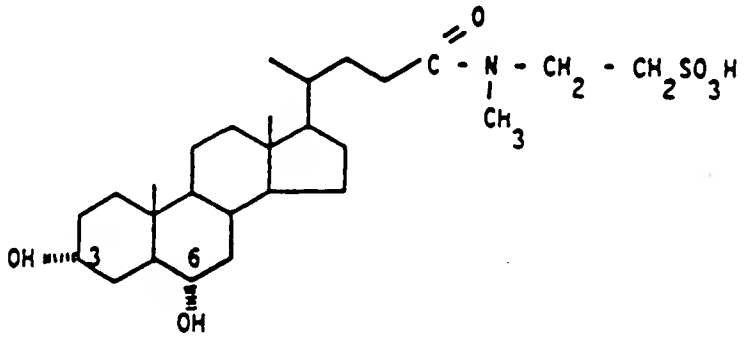

(I)

(57) Abstract

N-methyl-taurohydeoxycholic acid of formula (I) obtained from hyodeoxycholic acid and N-methyltaurine. The compound of formula (I) is endowed with advantageous therapeutic properties for the treatment of digestive apparatus pathologies.

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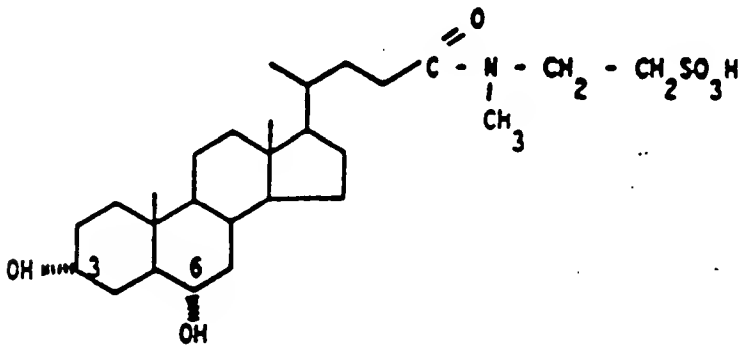
BILE ACID DERIVATIVE AND USE THEREOF IN THERAPY

The present invention relates to the N-methyl-taurohyodeoxycholic acid of formula (I)



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The present invention further relates to a process for the preparation of the compound (I) and to pharmaceutical compositions containing it.

Hyodeoxycholic acid (HDCA) is a natural bile salt which is present in pig bile. Hyodeoxycholic acid purification is described in US 2745849 and in US 3006927.

Recently, it has been proved that HDCA-taurine conjugate, namely taurohyodeoxycholic acid (EP 0293751) is potentially useful both in dissolving cholesterol calculi in man (Gastroenterology 100:A308, 1991) and in the promotion of bile secretion and desaturation. (Hepatology 14; 294A, 1991; Gastroenterology, May 1992).

Conjugation of other bile acids with N-methyl-taurine is well known (Hepatology, 11, 989-96, 1990; J. Lipid Res. 27, 742-52, 1986) as an effective mean to obviate the problem of deconjugation by colon bacteria. To date experimental results on N-methyltauro-

conjugates of bile acids do not anyway allow to draw final conclusions about the general validity of such an expedient to the purpose of the development of new therapeutic agent.

It has now been found that hyodeoxycholic acid (N-M-THDCA) is endowed with advantageous properties, which can be resumed as follows:

1 - Effective intestinal absorption mainly by active mechanism in the terminal ileum.

2 - Very good liver uptake and lack of hepatic biotransformation and/or metabolisation.

3 - Prompt and effective secretion into bile, without needing any conjugation phase, the molecule being already present in the "active" form, i.e. ready for the bile secretion.

4 - Remarkable choleretic and cholagogue activity.

5 - N-M-THDCA, similarly to the natural non N-methylated compound, has hydrophilic properties, which are intermediate between tauroursodeoxycholic and taurocholic acid ones.

6 - Absence of membrane toxicity, due to its hydrophilic character and to the poor detergent power.

7 - Reduced or absent intestinal deconjugation, due to the above cited resistance to bacterial enzymatic hydrolysis. This further avoids the compound to be 6-α-hydroxylated, therefore it is not transformed into lithocholic acid.

8 - Absence of hepatic and renal glucuronation as a direct consequence of the conjugation in the biologically stable form with N-M-Tau, which

prevents the elimination of the molecule through the renal pathway, as instead occurs for non conjugated hyodeoxycholic acid (J. Lipid Res. 24: 604-613, 1983).

The compound of the present invention can be prepared starting from hyodeoxycholic acid and N-methyltaurine, by using conventional techniques for the synthesis of bile acid amides; see for example Arkiv. Kemi 8, 331, 1955 and J. Lipid Res. 18: 400-407, 1967. Examples of these methods are the ones based on the use of mixed anhydrides, on the formation of bile acid azides and the ones providing the use of various coupling substances such as quinoline derivatives.

If desired, N-methyl-taurohyodeoxycholic acid can be salified with non toxic and pharmaceutically compatible cations.

Pharmacological tests proved that the compound of the invention can promote bile secretion of lipids, particularly cholesterol, in stable form, because of the formation of an abundant liquid-crystalline phase in the bile, which avoids any form of precipitation in the solid crystalline form of cholesterol monohydrate.

N-M-THDCA further resulted endowed with a powerful membrane protecting activity, due to its intrinsic hydrophilicity. In particular, N-M-THDCA is capable of protecting cells (and in particular hepatocytes) both from toxic-detergent action of endogen hydrophilic bile salts, and from toxic or viral agents.

N-M-THDCA has further shown a more prolonged pharmacological effect than natural bile acids.

From the above, it is evident that the compound of

the present invention can advantageously be used in human therapy, with the following indications:

1 - Prevention and therapy of gall-stones and biliary dyspepsia.

2 - Therapy of acute and chronic hepatopathy, including those of toxic and viral origin and those of cholestatic origin, both primary and secondary.

3 - Therapy of malabsorption, both primary and secondary.

4 - Therapy of gastropathy from alkaline biliary reflux.

5 - Therapy of hyperlipidemiae, both primary and secondary.

For the foreseen therapeutic uses, N-M-THDCA acid can be formulated into suitable pharmaceutical composition, by means of well known excipients and techniques, such as described for example in "Remington's Pharmaceutical Sciences Handbook", Mack Pub. Co. USA, XVII ed. Orally administrable compositions, such as capsules, tablets, sugar coated pills, sachets, extemporary solutions or suspensions, syrups, controlled release compositions, and the like are preferred. Daily mean posology will be established by the physician based on several parameters (patient's weight, sex and age, severity and stage of pathology), but as a general rule it will range between 100 and 1500 mg, optionally divided into 2 to 4 administrations. The following example further illustrates the invention.

EXAMPLE

1,035 mg of N-methyltaurine (N-M-Tau) into 1.8 ml of triethylamine were added under continous stirring to a solution containing 2,500 mg of N-methyl-taurohyodeoxycholic acid (HDCA) and 2.5 g of N-ethoxy-carbonyl-2-ethoxy-1,2-dihydroquinoline as coupling agent, dissolved into 15 ml of dimethylformamide. The resulting suspension was subsequently heated to 80-90°C for 40 minutes, to give a clear solution, always under stirring.

After cooling down to 5°C, the so obtained triethylammonium salt of taurohydeoxycholic acid was purified by slowly adding 150 ml of anhydrous diethylether, kept at 0°C, to the dimethylformamide solution.

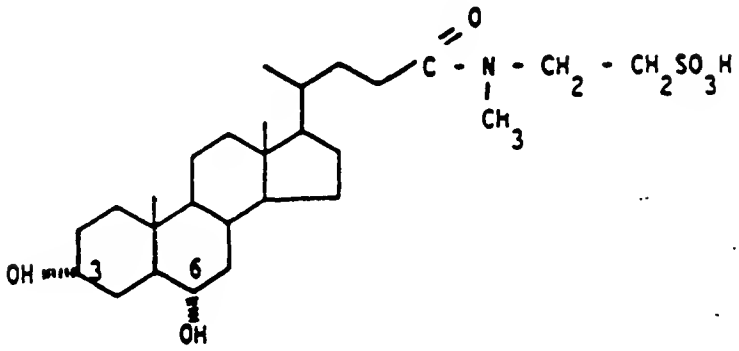
This produced an amorphous precipitate, containing the triethylammonium salt of N-M-THDCA, which, after repeated washings with 100 ml portions of anhydrous diethylether, was redissolved into 50 ml of methylene chloride and filtered.

The N-M-THDCA sodium salt was obtained by adding the same volume of 2N NaOH to the methylene chloride solution, then by precipitating repeatedly the salt with 100 ml of anhydrous ethyl acetate and filtering the pellet. N-M-THDCA was obtained by saturating the salt solution in methylene chloride with gaseous hydrochloric acid.

N-M-THDCA was obtained with a yield higher than 85% and in a chromatographically pure form, as proved by reverse phase HPLC and TLC.

CLAIMS

1. N-methyl-taurohyodeoxycholic acid of formula (I)



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and pharmaceutically acceptable salts thereof with non toxic cations.

2. The use of N-methyl-taurohyodeoxycholic acid for the manufacture of a medicament for the treatment of hepatopathies, gall-stones and biliary dyspepsia, malabsorption syndromes, gastropathy from alkaline biliary reflux, hyperlipidemiae.

3. Pharmaceutical compositions containing the compound of claim 1 or a non toxic salt thereof.

INTERNATIONAL SEARCH REPORT

Inte mal Application No
PCT/EP 93/01874

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C07J41/00 A61K31/575

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classificaton system followed by classification symbols)
IPC 5 C07J A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	FALK SYMPOSIUM vol. 42 , 1985 , LANCASTER, GB pages 199 - 204 E. H. MOSBACH ET AL 'Effect of Bile Acids and Bile Acid Analogs on Cholesterol Metabolism in the Hamster' see page 200, line 44 - page 203, line 43 see in particular page 203, lines 26-28 --- -/--	1-3

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

* Special categories of cited documents :

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9 November 1993	26. 11. 93

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CHEMICAL ABSTRACTS, vol. 115, no. 3, 22 July 1991, Columbus, Ohio, US; abstract no. 22019, J. MALVISI ET AL 'Pharmacodynamics of Taurohyodeoxycholic Acid in Rats and Mice: Effects on Hyperlipemia, Hypercholesterolemia, Frequency of Gallstones and Steatosis from High Cholesterol Diets' page 60 ;column 2 ; see abstract & ACTA TOXICOLOGICA ET THERAPEUTICA vol. 11, no. 3 , 1990 , PARMA, IT pages 205 - 214 ---	1-3
A	HEPATOLOGY vol. 11, no. 6 , 1990 , BALTIMORE, US pages 989 - 996 M. ANGELLOTTI ET AL 'Prevention of Ursodeoxycholate Hepatotoxicity in the Rabbit by Conjugation with N-methyl Amino Acids' see the whole document ---	1-3
A	JOURNAL OF BIOLOGICAL CHEMISTRY vol. 259, no. 24 , 25 December 1984 , BALTIMORE, MD US pages 15035 - 15039 A. K. BATTA ET AL 'Substrate Specificity f Cholylglycine Hydrolase for the ydrolysis of Bile Acid Conjugates' see page 15038; table V -----	1-3